Original Research Article

Preliminary Phytochemical and Antioxidant Activity of the Whole Plant of *Oldenlandia Corymbosa* Linn

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ABSTRACT

The *in vitro* antioxidant properties of the whole plant were studied in DPPH and NO models. The antioxidant study has been carried out with aqueous and alcoholic extracts. Both the extracts of the plant shows significant antioxidant properties. The plant was also analysed for the presence of various secondary metabolites by performing preliminary phytochemical screening, which shows the presence of many constituents including phenolic compounds and flavonoids. The antioxidant capacity attributed to the plant extract could be due to the presence of phenolic compounds and flavonoids.

Keywords: Antioxidants, DPPH, NO, Phenolic compounds, Flavonoids

INTRODUCTION

Cultivation, conservation and preservation of medicinal plants protect the biological diversity of the world. Even though, the major area of the earth's surface is covered with medicinal plants, it has not been explored completely for the search of medicinally active ingredients. Hence we have to give maximum priority in conserving medicinal plants. Most of the plants are considered as a source of drugs and the secondary metabolite present in them serves as a molecule for drug optimisation and renders intermediates which can be utilised in the production of semi-synthetic drugs.

Oldenlandia corymbosa (L.) (Rubiaceae) is a weedy annual herb, found throughout India. It is commonly known as 'Parppatakapullu' in traditional medicine of Kerala. The plant is known to clear heat and toxins, activate blood circulation, promote diuresis and relieve stranguria. A scrutiny of literature revealed some notable pharmacological activities of the plant such as hepatoprotective, cytotoxic, oxytocic and anti-malarial activity.^[1]

MATERIALS AND METHODS Plant material

The whole plants were collected from the hilly areas of Pariyaram, Kannur district of Kerala state in the month of September 2017. Its botanical identity was confirmed and then shade dried and the specimen bearing voucher No. OC (Wp) 01 has been deposited in the department of Pharmacognosy, Academy of Pharmaceutical Sciences, Pariyaram Medical College, Kannur district, Kerala state.

PRELIMINARY PHYTOCHEMICAL SCREENING ^[2,3]

Successive solvent extraction

Petroleum ether extract: The pulp obtained from ripe fruit (50g) was extracted with P.L.Rajagopal et al. Preliminary Phytochemical and Antioxidant Activity of the Whole Plant of Oldenlandia Corymbosa Linn

petroleum ether by hot extraction process (soxhlet) for 6 hr. After completion of extraction, the solvent was removed by distillation and concentrated.

Benzene extract: The marc left after petroleum ether extraction was dried and extracted with benzene by hot extraction process (soxhlet) for 6hr. After completion of extraction, the solvent was removed by distillation and concentrated.

Chloroform extract: The marc left after benzene extraction was dried and extracted with chloroform by hot extraction process (soxhlet) for 6hr. After completion of extraction, the solvent was removed by distillation and concentrated.

Acetone extract: The marc left after chloroform extraction was dried and extracted with acetone by hot extraction process (soxhlet) for 6hr. After completion of extraction, the solvent was removed by distillation and concentrated.

Methanolic extract: The marc left after acetone extraction was dried and extracted with (95%) methanol by hot extraction process (soxhlet) for 6hr. After completion of extraction, the solvent was removed by distillation and concentrated.

Aqueous extract: The marc left after methanol extraction was dried and extracted with distilled water by maceration process for 7 days. After completion of extraction, the solvent was removed by evaporation and concentrated. The above extracts were used for phytochemical studies.

ANTIOXIDANT STUDIES

Reduction of 1, 1- Diphenyl- 2-PicrylHydrazyl (DPPH) free radical ^[4]

The antioxidant reacts with stable free radical, DPPH and converts it to 1, 1diphenyl-2-picryl hydrazine. The ability to scavenge the free radical, DPPH was measured in the absorbance at 517 nm. To the 1ml of various concentrations of methanolic and aqueous extracts in a test tube, 1ml of solution of DPPH 0.1 mM was added to the test tube. An equal amount of methanol and DPPH were added to the control. Ascorbic acid was used as the standard for comparison. After 20 minutes incubation in the dark, absorbance was recorded at 517 nm. Experiment was performed in triplicate.

% Scavenging = $\frac{\text{Control} - \text{Test}}{\text{Control}} \ge 100$

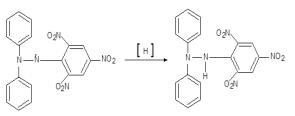


Fig. 1 Reduction of DPPH free radical

Nitric Oxide Scavenging Activity^[5]

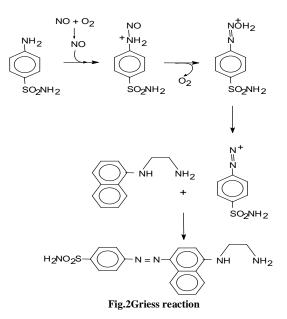
Nitric oxide is a very unstable species under aerobic condition. It reacts with O_2 to produce the stable product nitrates and nitrite through intermediates NO_2 , N_2O_4 and N_3O_4 . It is estimated by using the Griess reagent. In the presence of test compound, which is a scavenger, the amount of nitrous acid will decrease. The extent of decrease will reflect the extent of scavenging, which is measured at 546 nm.

Griess reagent preparation can be done by preparing and mixing of solution A and Solution B. Solution A can be prepared by adding 1% sulphanilamide in 5% ortho phosphoric acid or 25% v/v hydrochloric acid and Solution B can be prepared by adding 0.01% napthyl ethylene diamine in distilled water. Finally Solution A and Solution B were mixed equal volumes within 12 hours of use.

Sodium nitroprusside5mM was prepared in phosphate buffer pH 7.4. To 1 ml of various concentrations of the extract, 0.3 ml of sodium nitroprusside was added in the test tubes. The test tubes were incubated at 25°C for 5hr. After 5hrs, 0.5ml of Griess reagent was added. The absorbance was measured at 546 nm. The experiment was performed in triplicate.

% Scavenging = $\frac{\text{Control} - \text{Test}}{\text{Control}} \ge 100$

P.L.Rajagopal et al. Preliminary Phytochemical and Antioxidant Activity of the Whole Plant of Oldenlandia Corymbosa Linn



RESULTS AND DISCUSSION

Table 1 Effect of alcoholic and aqueous extracts of Oldenlandia corymbosa on DPPH scavenging

Sl.No.	Conc.	Ascorbic acid		Aqueous extract		Alcoholic extract	
	µg/ml	(Standard)				(Methanolic)	
		Abs	% Sca.	Abs.	% Sca.	Abs	% Sca.
1	5	0.976	15.30	0.950	0.59	0.979	2.79
2	10	0.899	30.64	0.878	3.85	0.910	6.53
3	15	0.657	39.39	0.811	5.19	0.856	20.11
4	25	0.590	47.25	0.799	10.75	0.799	35.99
5	50	0.157	59.39	0.745	21.63	0.650	49.24
6	100	0.097	68.44	0.650	35.99	0.599	57.65
7	250	0.065	74.97	0.544	47.22	0.410	69.30
8	500	0.043	89.60	0.442	59.55	0.220	75.24
9	1000	0.029	99.21	0.329	70.20	0.115	85.70
10	Control	0.997		0.997		0.954	

Plants are the major source for dietary fibers. The study done on medicinal plants and vegetables strongly supports the idea that plant constituents with antioxidant activity are capable of exerting protective effects against oxidative stress in biological systems. ^[6] Free radical production occurs continuously in cells as a part of normal cellular function. ^[7] However, excess free

radical production originating from endogenous or exogenous sources might play a role in many diseases. ^[7] Preliminary phytochemical investigation of the plant shows the presence of alkaloids, glycosides, flavonoids, carbohydrates and phenolic compounds in both aqueous as well as alcoholic fractions.

Sl.No.	Conc. µg/ml	Ascorbic acid (Standard)		Aqueous extract		Alcoholic extract (Methanolic)	
		Abs	% Sca.	Abs	% Sca.	Abs	% Sca.
1	5	0.797	7.40	0.715	2.50	0.831	3.94
2	10	0.651	9.19	0.693	6.99	0.709	6.21
3	15	0.577	13.50	0.614	15.20	0.641	19.41
4	25	0.420	22.74	0.550	29.39	0.611	20.15
5	50	0.359	41.65	0.493	46.50	0.534	37.19
6	100	0.275	59.35	0.379	53.21	0.497	49.22
7	250	0.199	67.41	0.316	69.75	0.310	56.30
8	500	0.097	79.35	0.201	76.10	0.297	69.27
9	1000	0.076	85.50	0.170	89.20	0.214	73.56
10	Control	0.825		0.769		0.715	

Table 2Effect of alcoholic and aqueous extracts Oldenlandia corymbosa on NO scavenging

P.L.Rajagopal et al. Preliminary Phytochemical and Antioxidant Activity of the Whole Plant of Oldenlandia Corymbosa Linn

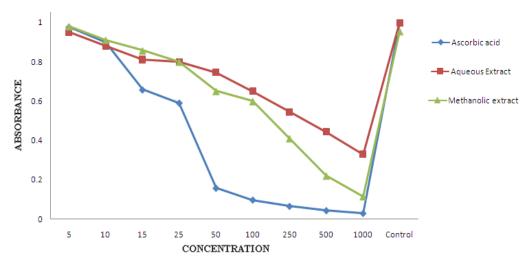


Fig 3. Effect of alcoholic and aqueous extracts of Oldenlandia corymbosa on DPPH scavenging

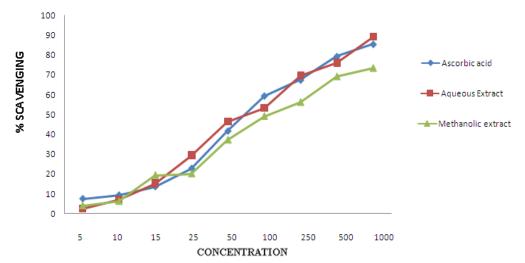


Fig 4. Effect of alcoholic and aqueous extracts of Oldenlandia corymbosa on DPPH scavenging

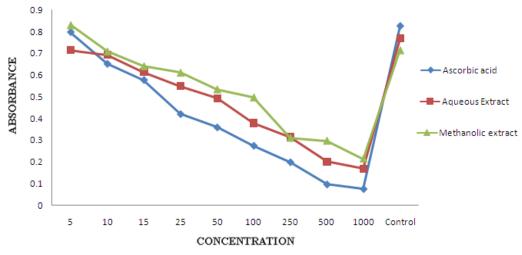


Fig 5.Effect of alcoholic and aqueous extracts Oldenlandia corymbosa on NO scavenging

P.L.Rajagopal et al. Preliminary Phytochemical and Antioxidant Activity of the Whole Plant of Oldenlandia Corymbosa Linn

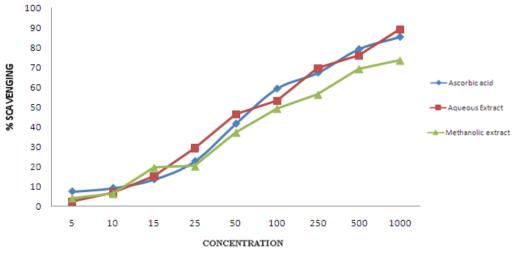


Fig. 6 Effect of alcoholic and aqueous extracts *Oldenlandia corymbosa* on NO scavenging

Flavonoids have been demonstrated to have anti-inflammatory, antiallergic, antiviral, antiaging and anti carcinogenic activity. The broad therapeutic effects of flavonoids can be largely attributed to [8] antioxidant properties. In plants, flavonoids serves as protectors against a wide variety of environmental stress while in humans, flavonoids appear to function as biological response modifiers. Antioxidants prevent free radicals induced tissue damage by preventing the formation of radicals, scavenging them or by promoting their decomposition.^[7] From our study it is very clear that the whole plant possess significant antioxidant activity in DPPH and NO scavenging in vitro models. Despite many plants being reported to have antioxidant potential by in vitro assays, only a few of these antioxidant activities have been confirmed or investigated in vivo.^[10] Several phytochemicals have been found to possess antioxidant activity within in vitro assays, however only a few of these have been shown to be therapeutically useful under in vivo conditions due to their interference with physiopharmacological process such as absorption, distribution, metabolism, storage and excretion.^[11] Thus an antioxidant can be defined as any substance that when present in low concentrations compared to that of an oxidisable substrate, significantly delays or inhibits the oxidation of that substrate.^[12] Hence in future the *in vivo* antioxidant capacity of the plant has to be carried out, which will be very helpful in developing a promising antioxidant herbal molecule.

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P.L.Rajagopal et al. Preliminary Phytochemical and Antioxidant Activity of the Whole Plant of Oldenlandia Corymbosa Linn

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